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<b>(54) Title:</b> ENHANCEMENT OF LACCASE REACTIONS  <b>(57) Abstract</b>  This invention relates to methods of oxidizing a substrate in the presence of a laccase or a laccase related enzyme and an enhancing agent. More specifically, the invention relates to a method of bleaching of dye in solutions, to a method of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, to a method of bleaching of lignin-containing material, in particular bleaching of pulp for paper production, to a method of treatment of waste water from pulp manufacturing, and to a method of enzymatic polymerization and/or modification of lignin or lignin containing material.		

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## ENHANCEMENT OF LACCASE REACTIONS

### TECHNICAL FIELD

This invention relates to activation of enzymes. More specifically, the invention relates to agents capable of enhancing the activity of laccases and related enzymes.

The invention also relates to methods of oxidizing a substrate in the presence of a laccase enzyme, and an enhancing agent. More specifically, the invention relates to a method of bleaching of dye in solutions, to a method of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, to a method of bleaching lignin-containing material, in particular bleaching of pulp for paper production, to a method of treatment of waste water from pulp manufacturing, and to a method of enzymatic polymerization and/or modification of lignin or lignin containing material.

### BACKGROUND ART

Laccases (E.C. 1.10.3.2) are enzymes that catalyse the oxidation of a substrate (an electron or hydrogen donor) with oxygen. Such enzymes are known from microbial, plant and animal origins, e.g. from fungi. They are typically copper proteins, i.e. they contain a copper atom or atoms as a prosthetic group.

Use of laccases has been suggested e.g. in bleaching of pulp for paper production, in treatment of waste water from pulp production, for improved bleaching in laundry detergents, for dye transfer inhibition during laundering, and for lignin modification, e.g. in particle board production.

The compound 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate), ABTS, supplied by Boehringer Mannheim, is a chromogenic substrate, and a common peroxidase and phenol oxidase assay agent. These enzymes catalyse the oxidation of

ABTS by hydrogen peroxide and dioxygen, respectively, producing a greenish-blue colour, which process may be monitored photometrically.

ABTS has been found to form a stable radical cation when oxidized by a laccase enzyme (polyphenol oxidase, EC 1.10.3.2), and has been proposed to act as a redox mediator for oxidation of non-phenolic lignin model compounds [Bourbonnais R, Paice M G; FEBS Lett (1990) 267 99-102].

Studies on demethylation and delignification of kraft pulp by a laccase enzyme in the presence of ABTS showed that the extent of partial demethylation by laccase was increased in the presence of ABTS [Bourbonnais, R. and Paice, M.G; Appl. Microbiol. Biotechnol. (1992) 36 823-827].

Certain oxidizable substrates e.g. metal ions and phenolic compounds such as 7-hydroxycoumarin (7HcM), vanillin (VAN), and p-hydroxybenzenesulfonate (pHBS), have been described as accelerators or enhancers, able to enhance bleaching reactions (cf. e.g. WO 92/18683, WO 92/18687, and Kato M and Shimizu S, Plant Cell Physiol. 1985 26 (7), pp. 1291-1301 (cf. Table 1 in particular), or Saunders B C, et al., Peroxidase, London, 1964, p. 141 ff).

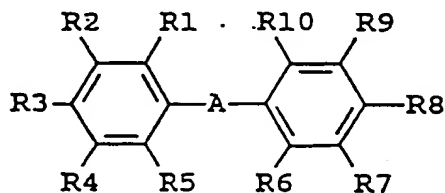
#### SUMMARY OF THE INVENTION

It has now surprisingly been found that organic chemical compounds consisting of at least two aromatic rings, of which aromatic rings at least one ring is substituted with one or more of the following atoms: nitrogen, oxygen, and sulfur, and which aromatic rings may furthermore be fused rings, are capable of enhancing the activity of laccase enzymes.

Accordingly, in its first aspect, the invention provides an agent for enhancing the activity of laccase enzymes, which agent is an organic chemical compound consisting of at least two aromatic rings, of which aromatic rings at least one ring is substituted with one or more of the following

atoms: nitrogen, oxygen, and sulfur; and which aromatic rings may furthermore be fused rings.

In a more specific aspect, the invention provides an agent for enhancing the activity of laccase enzymes, which agent is an organic chemical compound of the general formula I:



in which general formula A represents a single bond, or one of the following groups:  $(-CR^{11}=CR^{15}-)_n$ ,  $(-CR^{11}=N-N=CR^{15}-)$ ,  $(-NR^{11}-)$ ,  $(-CR^{12}=N-)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{15}-)$ ,  $(-NR^{11}-CR^{12}=N-)_n$ ,  $(-CR^{12}=N-NR^{11}-)$ ,  $(-NR^{11}-CR^{12}=CR^{13}-)$ ,  $(-N=N-)$ , in which groups n represents an integer of from 1 to 6; or A represents carbon, carbonyl, nitrogen, sulfur, oxygen, selenium, or phosphor, which carbon, phosphor and nitrogen may be unsubstituted or substituted with a substituent group  $R^{11}$ ;

and in which general formula the substituent groups  $R^1$ - $R^{13}$  and  $R^{15}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy, sulfandiyl, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{14}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ;

which substituent group  $R^{14}$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and

salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyll, pyrrolidino, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be  
5 unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;  
10 and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof,  
15 and sulfamoyl;

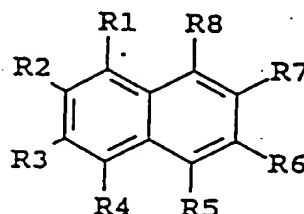
or in which general formula R<sup>5</sup> and R<sup>6</sup> may together form a group -B-, in which B represents a single bond, the group (-CH=CH-)<sub>n</sub>, or the group (-CH=N-)<sub>n</sub>, in which groups n represents an integer of from 1 to 6; or B represents carbon,  
20 nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group R<sup>14</sup> as defined above;

or in which general formula two of the substituent groups R<sup>1</sup>-R<sup>10</sup> may together form a group -C-, in which C represents any of the following groups: (-CHR<sup>11</sup>-N=N-), (-CH=CH-)<sub>n</sub>,  
25 (-CH=N-)<sub>n</sub>, in which groups n represents an integer of from 2 to 4, and in which groups R<sup>11</sup> is a substituent group as defined above;

or in which general formula R<sup>5</sup> and R<sup>12</sup>, and/or R<sup>6</sup> and  
30 R<sup>13</sup>, when n in the above formula being 1, may together form a group -D-, in which D represents the groups: (-CHR<sup>11</sup>-), (-NR<sup>11</sup>-), (-CR<sup>11</sup>=CR<sup>15</sup>-), (-CR<sup>11</sup>=N-), (-N=CR<sup>11</sup>-), (-O-), (>C=O) or (-S-), and in which groups R<sup>11</sup> and R<sup>15</sup> are substituent groups as defined above.

35 In another specific aspect, the invention provides an agent for enhancing the activity of a laccase enzyme, which

agent is an organic chemical compound of the general formula II:



in which the substituent groups  $R^1$ - $R^8$ , which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^9$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ;

which substituent group  $R^9$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_3$ -alkyl,  $C_1$ - $C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups R<sup>1</sup>-R<sup>8</sup> may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub>, (-N=CR<sup>9</sup>-NR<sup>10</sup>-) or (-N=N-CR<sup>9</sup>-), in which groups n represents an integer of from 1 to 3, R<sup>9</sup> is a substituent group as defined above and R<sup>10</sup> is defined as R<sup>9</sup>.

In another aspect, the invention provides a method for oxidizing a substrate with a laccase or a laccase related enzyme, in the presence of oxygen and in the presence of an enhancing agent of the invention.

In a more specific aspect, the invention provides a method for bleaching dye in solutions by treatment with a laccase or a laccase related enzyme in the presence of oxygen in the presence of an enhancing agent of the invention.

In another specific aspect, the invention provides a method of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the method comprising treatment of the wash liquor with a laccase or a laccase related enzyme in the presence of oxygen and in the presence of an enhancing agent of the invention.

In another aspect, the invention provides a method of bleaching lignin-containing material, in particular bleaching of pulp for paper production, the method comprising treatment of the lignin or lignin containing material with a laccase or a laccase related enzyme in the presence of a source of oxygen and in the presence of an enhancing agent of the invention.

In a further aspect, the invention provides a method of enzymatic polymerization and/or modification of lignin or



lignin containing material, the method comprising treatment of the lignin or lignin containing material with a laccase or a laccase related enzyme in the presence of a source of oxygen and in the presence of an enhancing agent of the invention.

5 In a yet further aspect, the invention provides a method of treatment of waste water, in particular waste water from pharmaceutical or chemical industry, e.g. waste water from dye manufacturing, from textile industry, or from pulp manufacturing, the method comprising treatment of the waste water  
10 with a laccase or a laccase related enzyme in the presence of a source of oxygen and in the presence of an enhancing agent of the invention.

In a particular aspect, the invention provides a detergent additive capable of inhibiting the transfer of a  
15 textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the detergent additive comprising an enzyme exhibiting laccase activity and oxygen and an enhancing agent of the invention.

In other aspects, the invention provides detergent  
20 additives and detergent compositions capable of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the detergent composition comprising an enzyme exhibiting laccase activity and oxygen and an enhancing agent of the invention.

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#### BRIEF DESCRIPTION OF DRAWINGS

The present invention is further illustrated by reference to the accompanying drawings, in which:

Fig. 1 shows the specific formulas of some enhancing agents of the invention (I) 6-hydroxy-2-naphtoic acid; (II) 7-methoxy-2-naphtol; (III) 7-amino-2-naphthalene sulfonic acid;  
30 (IV) 5-amino-2-naphthalene sulfonic acid; (V) 1,5-diamino-naphthalene; (VI) 7-hydroxy-1,2-naphthimidazole; (VII) 10-methylphenothiazine; (VIII) 10-phenothiazine-propionic acid; (IX) N-hydroxysuccinimide-10-phenothiazine-propionate; (X)

benzidine; (XI) 3,3'-dimethylbenzidine; (XII) 3,3'-dimethoxybenzidine; (XIII) 3,3',5,5'-tetramethylbenzidine; (XIV) 4'-hydroxy-4-biphenylcarboxylic acid; (XV) 4-amino-4'-methoxystilbene; (XVI) 4,4'-diaminostilbene-2,2'-disulfonic acid; (XVII) 4,4'-diaminodiphenylamine; (XVIII) 2,7-diaminofluorene; (XIX) 4,4'-dihydroxy-biphenylene; (XX) triphenylamine; (XXI) 10-ethyl-4-phenothiazinecarboxylic acid; (XXII) 10-ethylphenothiazine; (XXIII) 10-propylphenothiazine; (XXIV) 10-isopropylphenothiazine; (XXV) methyl-10-phenothiazinepropionate; (XXVI) 10-phenylphenothiazine; (XXVII) 10-allylphenothiazine; (XXVIII) 10-phenoxazinepropionic acid; (XXIX) 10-(3-(4-methyl-1-piperazinyl)propyl)phenothiazine; (XXX) 10-(2-pyrrolidinoethyl)phenothiazine; (XXXI) 10-methylphenoxazine; (XXXII) iminostilbene; (XXXIII) 2-(p-aminophenyl)-6-methylbenzothiazole-7-sulfonic acid; (XXXIV) N-benzylidene-4-biphenylamine; (XXXV) 5-amino-2-naphthalenesulfonic acid; (XXXVI) 7-methoxy-2-naphtol; (XXXVII) 4,4'-dihydroxybenzophenone; (XXXVIII) N-(4-(dimethylamino)benzylidene)-p-anisidine; (XXXIX) 3-methyl-2-benzothiazolinone(4-(dimethylamino)benzylidene)hydrazine; (XL) 2-acethyl-10-methylphenothiazine; (XLI) 10-(2-hydroxyethyl)phenothiazine; (XLII) 10-(2-hydroxyethyl)phenoxazine; (XLIII) 10-(3-hydroxypropyl)phenothiazine; (XLIV) 4,4'-dimethoxy-N-methyl-diphenylamine; (XLV) vanillin azine.

Fig. 2 shows the bleaching of Direct Blue 1 with bilirubin oxidase and 10-methylphenothiazine (MPT) ( $\square$  0  $\mu$ M MPT;  $\blacklozenge$  2.5  $\mu$ M MPT;  $\blacksquare$  5.0  $\mu$ M MPT;  $\diamond$  10.0  $\mu$ M MPT).

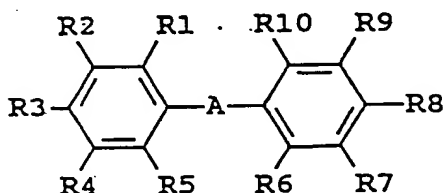
## DETAILED DISCLOSURE OF THE INVENTION

### The Enhancing Agent

The present invention relates to the use of chemical compounds for enhancing the activity of laccase enzymes. Accordingly, the invention provides an agent capable of enhancing the effect of a laccase enzyme in the following termed enhancing agent.

The enhancing agent of the invention is an organic chemical compound consisting of at least two aromatic rings, of which aromatic rings at least one ring is substituted with one or more nitrogen, oxygen, and/or sulfur atoms, and which aromatic rings may furthermore be fused rings.

In a more preferred embodiment, the enhancing agent of the invention is an organic chemical compound of the general formula I:



in which general formula A represents a single bond, or one of the following groups:  $(-CR^{11}=CR^{15}-)_n$ ,  $(-CR^{11}=N-N=CR^{15}-)$ ,  $(-NR^{11}-)$ ,  $(-CR^{12}=N-)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{15}-)$ ,  $(-NR^{11}-CR^{12}=N-)_n$ ,  $(-CR^{12}=N-NR^{11}-)$ ,  $(-NR^{11}-CR^{12}=CR^{13}-)$ ,  $(-N=N-)$ , in which groups n represents an integer of from 1 to 6; or A represents carbon, carbonyl, nitrogen, sulfur, oxygen, selenium, or phosphor, which carbon, phosphor and nitrogen may be unsubstituted or substituted with a substituent group  $R^{11}$ ;

and in which general formula the substituent groups  $R^1$ - $R^{13}$  and  $R^{15}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy, sulfandiyl, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{14}$ ; and which phenyl may furthermore be unsubstituted or

substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted  
5 with one or more substituent groups  $R^{14}$ ;

which substituent group  $R^{14}$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, aminoalkyl, piperidino,  
10 piperazinyl, pyrrolidino,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals:  
15 hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the  
20 following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

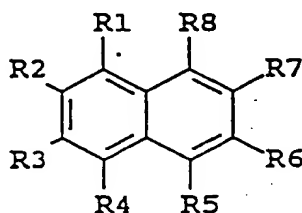
or in which general formula  $R^5$  and  $R^6$  may together form a group -B-, in which B represents a single bond, the  
25 group  $(-CH=CH-)_n$ , or the group  $(-CH=N-)_n$ , in which groups n represents an integer of from 1 to 6; or B represents carbon, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group  $R^{14}$  as defined above;

30 or in which general formula two of the substituent groups  $R^1$ - $R^{10}$  may together form a group -C-, in which C represents any of the following groups:  $(-CHR^{11}-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$ , in which groups n represents an integer of from 2 to 4, and in which groups  $R^{11}$  is a substituent group as defined  
35 above;

or in which general formula  $R^5$  and  $R^{12}$ , and/or  $R^6$  and  $R^{13}$ , when  $n$  in the above formula being 1, may together form a group -D-, in which D represents the groups: (-CHR<sup>11</sup>-), (-NR<sup>11</sup>-), (-CR<sup>11</sup>=CR<sup>15</sup>-), (-CR<sup>11</sup>=N-), (-N=CR<sup>11</sup>-), (-O-), (>C=O) or (-S-), and  
 5 in which groups  $R^{11}$  and  $R^{15}$  are substituent groups as defined above.

In particular embodiments, the enhancing agent is 2-(p-aminophenyl)-6-methylbenzothiazole-7-sulfonic acid, N-(4-(dimethylamino)benzylidene)-p-anisidine, 3-methyl-2-benzo-  
 10 thiazolinone (4-(dimethylamino)benzylidene)hydrazone, vanillin azine.

In another preferred embodiment, the enhancing agent of the invention is an organic chemical compound of the general formula II:



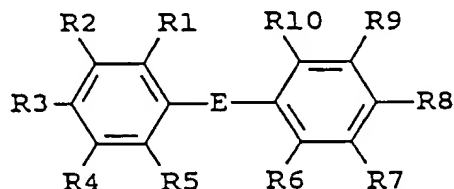
15 in which the substituent groups  $R^1$ - $R^8$ , which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -  
 20 alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^9$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -

alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>;

5 which substituent group R<sup>9</sup> represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be  
 10 unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;  
 15 and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof,  
 20 and sulfamoyl;

or in which general formula two of the substituent groups R<sup>1</sup>-R<sup>8</sup> may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub>, (-N=CR<sup>9</sup>-NR<sup>10</sup>-) or (-N=N-CR<sup>9</sup>-), in which groups n represents an integer of from 1 to 3, R<sup>9</sup> is a substituent group as  
 25 defined above and R<sup>10</sup> is defined as R<sup>9</sup>.

In a more specific embodiment, the enhancing agent of the invention is an organic chemical compound of the following formula:



in which formula E represents a single bond, a carbonyl group or one of the following groups:  $(-\text{CH}=\text{CH}-)_n$ ,  $(-\text{CH}=\text{N}-)_n$  or  $(-\text{NR}^{11}-)$ , in which n represents an integer from 1 to 2. The substituents groups  $\text{R}^1$ - $\text{R}^{11}$  may be identical or different, independently being one of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $\text{C}_1$ - $\text{C}_{14}$ -alkyl,  $\text{C}_1$ - $\text{C}_5$ -alkoxy, carbonyl- $\text{C}_1$ - $\text{C}_5$ -alkyl, aryl- $\text{C}_1$ - $\text{C}_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $\text{R}^{14}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $\text{R}^{14}$ ; and which  $\text{C}_1$ - $\text{C}_{14}$ -alkyl,  $\text{C}_1$ - $\text{C}_5$ -alkoxy, carbonyl- $\text{C}_1$ - $\text{C}_5$ -alkyl, and aryl- $\text{C}_1$ - $\text{C}_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $\text{R}^{14}$ ;

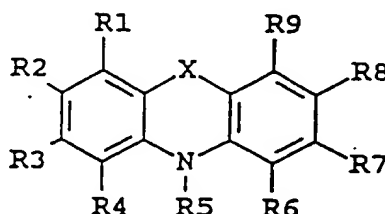
which substituent group  $\text{R}^{14}$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $\text{C}_1$ - $\text{C}_5$ -alkyl,  $\text{C}_1$ - $\text{C}_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $\text{C}_1$ - $\text{C}_3$ -alkyl,  $\text{C}_1$ - $\text{C}_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $\text{C}_1$ - $\text{C}_5$ -alkyl, and  $\text{C}_1$ - $\text{C}_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which specific formula two of the substituent groups  $\text{R}^5$  and  $\text{R}^6$  may together form a group -B-, in which B represents the groups:  $(-\text{CH}=\text{N}-)_n$ ,  $(-\text{CH}=\text{CH}-)$  or  $(-\text{CHR}^{14}-)$  in which

groups  $n$  represents an integer of from 1 to 2 and  $R^{14}$  is a substituent group as defined above.

In particular embodiments, the enhancing agent is 4-amino-4'-methoxystilbene, 4,4'-diaminostilbene-2,2'-disulfonic acid, iminostilbene, 4,4'-dihydroxybenzophenone, N-benzylidene-4-biphenylamine, 4,4'-diaminodiphenylamine, 4,4'-dimethoxy-N-methyl-diphenylamine, 2,7-diaminofluorene, triphenylamine.

In another specific embodiment, the enhancing agent may be described by the following formula:



10 in which formula X represents one of the following groups: (-O-), (-S-), (-NR<sup>15</sup>-), (-CHR<sup>15</sup>-), (>C=O), (-CH=CH-), (-CH=N-) and the substituent groups R<sup>1</sup>-R<sup>9</sup> and R<sup>15</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and  
 15 esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>10</sup>; and  
 20 which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>10</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or  
 25 more substituent groups R<sup>10</sup>;

which substituent group R<sup>10</sup> represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, aminoalkyl, piperidino,



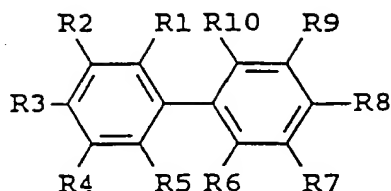
piperaziny, pyrrolidino, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups R<sup>1</sup>-R<sup>8</sup> may together form a group -B-, in which B represents any of the following the groups: (-CHR<sup>10</sup>-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub> or (-N=CR<sup>10</sup>-NR<sup>11</sup>-), in which groups n represents an integer of from 1 to 3, R<sup>10</sup> is a substituent group as defined above and R<sup>11</sup> is defined as R<sup>10</sup>.

In particular embodiments, the enhancing agent is 10-methylphenothiazine, 10-phenothiazine-propionic acid, N-hydroxysuccinimide-10-phenothiazine-propionate or 10-ethyl-4-phenothiazine-carboxylic acid, 10-ethylphenothiazine, 10-propylphenothiazine, 10-isopropylphenothiazine, methyl-10-phenothiazinepropionate, 10-phenylphenothiazine, 10-allylphenothiazine, 10-(3-(4-methyl-1-piperaziny)propyl)phenothiazine, 10-(2-pyrrolidinoethyl)phenothiazine, 2-acetyl-10-methylphenothiazine, 4-carboxy-10-phenothiazine, 10-methylphenoxazine, 10-ethylphenoxazine, 10-phenoxazine-propionic acid, 4-carboxy-10-phenoxazine-propionic acid, 10-(2-hydroxyethyl)phenothiazine, 10-(2-hydroxyethyl)phenoxazine or 10-(3-hydroxypropyl)phenothiazine.

In another specific embodiment, enhancing agent is a biphenyl derivative of the following formula:

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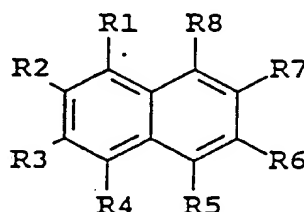
in which the substituent groups  $R^1$ - $R^{10}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{11}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{11}$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{11}$ ;

which substituent group  $R^{11}$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_3$ -alkyl,  $C_1$ - $C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl.

In particular embodiments, the enhancing agent is benzidine, 3,3'-dimethylbenzidine, 3,3'-dimethoxybenzidine,

3,3',5,5'-tetramethylbenzidine, 4'-hydroxy-4-biphenylcarboxylic acid, or 4,4'-dihydroxybiphenylene.

In another specific embodiment, the enhancing agent is a naphthalene derivative of the following formula:



5 in which the substituent groups R<sup>1</sup>-R<sup>8</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-  
10 alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>9</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-  
15 alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>;

which substituent group R<sup>9</sup> represents any of the  
20 following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be

unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups R<sup>1</sup>-R<sup>8</sup> may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub>, (-N=CR<sup>9</sup>-NR<sup>10</sup>-) or (-N=N-CR<sup>9</sup>-), in which groups n represents an integer of from 1 to 3, R<sup>9</sup> is a substituent group as defined above and R<sup>10</sup> is defined as R<sup>9</sup>.

In particular embodiments, the enhancing agent is 6-hydroxy-2-naphtoic acid, 7-methoxy-2-naphtol, 7-amino-2-naphthalene sulfonic acid, 5-amino-2-naphthalene sulfonic acid, 1,5-diaminonaphthalene, 7-hydroxy-1,2-naphthimidazole, 5-amino-2-naphthalenesulfonic acid, or 7-methoxy-2-naphtol.

The enhancing agent of the invention may be in free form or in the form of an addition salt.

#### 25 Methods of Oxidizing a Substrate

In another aspect, the invention provides a method of oxidizing a substrate with a laccase enzyme and in the presence of an enhancing agent.

The enhancing agent may be present in free form or in the form of an addition salt.

The enhancing agent may be present in concentrations of from 0.01 to 500 μM, more preferred 0.1 to 250 μM, most preferred 0.5 to 100 μM.

Molecular oxygen from the atmosphere will usually be present in sufficient quantity.

### Laccase and Related Enzymes

The enhancing agents of the invention are agents capable of enhancing the activity of laccases and laccase related enzymes. In the context of this invention, laccases and  
5 laccase related enzymes contemplate any laccase enzyme comprised by the enzyme classification EC 1.10.3.2, any catechol oxidase enzyme comprised by the enzyme classification EC 1.10.3.1, any bilirubin oxidase enzyme comprised by the enzyme classification EC 1.3.3.5 or any monophenol monooxygenase  
10 enzyme comprised by the enzyme classification EC 1.14.99.1.

Preferably, the laccase employed in the method of the invention is derived from a fungi such as Trametes, e.g. T. villosa or T. versicolor, Collybia, Fomes, Lentinus, Pleurotus, Rhizoctonia, e.g. R. solani, Aspergillus, Neurospora, Podospora,  
15 Phlebia, e.g. P. radiata (WO 92/01046), Coriolus, e.g. C. hirsitus (JP 2-238885), Myceliophthora, e.g. M. thermophila or Botrytis. Bilirubin oxidase may preferably be derived from Myrothecium, e.g. M. verrucaria.

The laccase or the laccase related enzyme may  
20 furthermore be one which is producible by a method comprising cultivating a host cell transformed with a recombinant DNA vector which carries a DNA sequence encoding said laccase as well as DNA sequences encoding functions permitting the expression of the DNA sequence encoding the laccase, in a  
25 culture medium under conditions permitting the expression of the laccase enzyme, and recovering the laccase from the culture.

### Determination of Laccase Activity (LACU)

Laccase activity is determined from the oxidation of  
30 syringaldazin under aerobic conditions. The violet colour produced is photometered at 530 nm. The analytical conditions are 19  $\mu$ M syringaldazin, 23.2 mM acetate buffer, pH 5.5, 30°C, 1 min. reaction time.

1 laccase unit (LACU) is the amount of enzyme that catalyses the conversion of 1.0  $\mu$ mole syringaldazin per minute at these conditions.

Determination of Bilirubin Oxidase Activity (Sigma Units)

5 1 bilirubin oxidase Sigma unit is the amount of enzyme that will oxidize 1.0  $\mu$ mole of bilirubin per minute at pH 8.4 and 30°C.

Industrial Applications

In a preferred embodiment, the method of the  
10 invention finds application for bleaching of dye in solutions.

In another embodiment, the method of the invention finds application for dye transfer inhibition, e.g. for treatment of dyed textiles (cf. e.g. WO 92/18687) or during laundering (cf. e.g. WO 91/05839).

15 Accordingly, in a specific embodiment, the invention provides a method for inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the method comprising treatment of the wash liquor with a laccase or a laccase  
20 related enzyme in the presence of an enhancing agent. The textile dye may be a synthetic dye such as an azo dye, or a natural or nature-identical dye.

In a third embodiment, the method of the invention finds application in bleaching of pulp for paper production.

25 Accordingly, the invention provides a method for bleaching of lignin-containing material, in particular bleaching of pulp for paper production, which method comprises treatment of the lignin or lignin containing material with a laccase or a laccase related enzyme in the presence of an  
30 enhancing agent of the invention.

In a fourth embodiment, the method of the invention finds application for lignin modification, e.g. in the manufacture of wood composites, e.g. wood fibre materials such as chipboards, fibre boards, or particle boards, or in the

manufacture of laminated wood products, such as laminated beams and plywood.

Accordingly, the invention provides a method for enzymatic polymerization and/or modification of lignin or lignin containing material, which method comprises treatment of the lignin or lignin containing material with a laccase or a laccase related enzyme in the presence of an enhancing agent of the invention.

In a fifth embodiment, the method of the invention finds application in treatment of waste water e.g. waste water from the chemical or pharmaceutical industry, from dye manufacturing, from dye-works, from the textile industry, or from pulp production (cf. e.g. US 4,623,465, or JP-A-2-31887).

In a more specific aspect, the invention provides a method for treatment of waste water from dye manufacturing, from dye-works, from textile industry, or from pulp manufacturing, the method comprising treatment of the waste water with a laccase or a laccase related enzyme in the presence of an enhancing agent of the invention.

#### 20 Detergent Compositions

According to the invention, the enhancing agent may be added as a component of a detergent composition. As such, it may be included in the detergent composition in the form of a detergent additive. The detergent composition as well as the detergent additive may additionally comprise one or more other enzymes conventionally used in detergents, such as proteases, lipases, amylases, cutinases, cellulases and peroxidases.

In a specific aspect, the invention provides a detergent additive. The enzymes may be included in a detergent composition by adding separate additives containing one or more enzymes, or by adding a combined additive comprising all of these enzymes. A detergent additive of the invention, i.e. a separated additive or a combined additive, can be formulated e.g. as granulates, liquids, slurries, etc. Preferred detergent additive formulations are granulates, in particular non-dusting

granulates, liquids, in particular stabilized liquids, slurries, or protected enzymes.

Non-dusting granulates may be produced, e.g., as disclosed in US 4,106,991 and 4,661,452 (both to Novo Industri A/S) and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethyleneglycol, PEG) with mean molar weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in patent GB 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Other enzyme stabilizers are well known in the art. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

The detergent composition of the invention may be in any convenient form, e.g. as powder, granules, paste or liquid. A liquid detergent may be aqueous, typically containing up to 70 % water and 0-30 % organic solvent, or nonaqueous.

The detergent composition comprises one or more surfactants, each of which may be anionic, nonionic, cationic, or zwitterionic. The detergent will usually contain 0-50 % of anionic surfactant such as linear alkylbenzenesulfonate (LAS), alpha-olefinsulfonate (AOS), alkyl sulfate (fatty alcohol sulfate) (AS), alcohol ethoxysulfate (AEOS or AES), secondary alkanesulfonates (SAS), alpha-sulfo fatty acid methyl esters, alkyl- or alkenylsuccinic acid or soap. It may also contain 0-40 % of nonionic surfactant such as alcohol ethoxylate (AEO or AE), carboxylated alcohol ethoxylates, nonylphenol ethoxylate, alkylpolyglycoside, alkyldimethylamineoxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, or



polyhydroxy alkyl fatty acid amide (e.g. as described in WO 92/06154).

The detergent composition may additionally comprise one or more other enzymes, such as amylases, lipases, cutinases, 5 proteases, cellulases and peroxidases.

The detergent may contain 1-65 % of a detergent builder or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepen- 10 taacetic acid (DTMPA), alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (e.g. SKS-6 from Hoechst). The detergent may also be unbuilt, i.e. essentially free of detergent builder.

The detergent may comprise one or more polymers. 15 Examples are carboxymethylcellulose (CMC), poly(vinylpyrrolidone) (PVP), polyethyleneglycol (PEG), poly(vinyl alcohol) (PVA), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

20 The detergent may contain a bleaching system which may comprise a  $H_2O_2$  source such as perborate or percarbonate which may be combined with a peracid-forming bleach activator such as tetraacetythylenediamine (TAED) or nonanoyloxybenzenesulfonate (NOBS). Alternatively, the bleaching system may comprise 25 peroxyacids of e.g. the amide, imide, or sulfone type.

The enzymes of the detergent composition of the invention may be stabilized using conventional stabilizing agents, e.g. a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid 30 derivative as e.g. an aromatic borate ester, and the composition may be formulated as described in e.g. WO 92/19709 and WO 92/19708.

The detergent may also contain other conventional detergent ingredients such as e.g. fabric conditioners in- 35 cluding clays, foam boosters, suds suppressors, anti-corrosion

agents, soil-suspending agents, anti-soil redeposition agents, dyes, bactericides, optical brighteners, or perfume.

The pH (measured in aqueous solution at use concentration) will usually be neutral or alkaline, e.g. 7-11.

5 Particular forms of detergent compositions within the scope of the invention include:

1) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

10	- linear alkylbenzenesulfonate (calculated as acid)	7 - 12%
	- alcohol ethoxysulfate (e.g. C <sub>12-18</sub> alcohol, 1-2 EO) or alkyl sulfate (e.g. C <sub>16-18</sub> )	1 - 4%
15	- alcohol ethoxylate (e.g. C <sub>14-15</sub> alcohol, 7 EO)	5 - 9%
	- sodium carbonate (as Na <sub>2</sub> CO <sub>3</sub> )	14 - 20%
	- soluble silicate (as Na <sub>2</sub> O, 2SiO <sub>2</sub> )	2 - 6%
	- zeolite (as NaAlSiO <sub>4</sub> )	15 - 22%
	- sodium sulfate (as Na <sub>2</sub> SO <sub>4</sub> )	0 - 6%
20	- sodium citrate/citric acid (as C <sub>6</sub> H <sub>5</sub> Na <sub>3</sub> O <sub>7</sub> /C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> )	0 - 15%
	- sodium perborate (as NaBO <sub>3</sub> ·H <sub>2</sub> O)	11 - 18%
	- TAED	2 - 6%
	- carboxymethylcellulose	0 - 2%
25	- polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG)	0 - 3%
	- enzymes	0 - 5%
30	- minor ingredients (e.g. suds suppressors, perfume, optical brightener, photobleach)	0 - 5%

2) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

	- linear alkylbenzenesulfonate (calculated as acid)	6 - 11%
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	- alcohol ethoxysulfate (e.g. C <sub>12-18</sub> alcohol, 1-2 EO) or alkyl sulfate (e.g. C <sub>16-18</sub> )	1 - 3%
5	- alcohol ethoxylate (e.g. C <sub>14-15</sub> alcohol, 7 EO)	5 - 9%
	- sodium carbonate (as Na <sub>2</sub> CO <sub>3</sub> )	15 - 21%
	- soluble silicate (as Na <sub>2</sub> O, 2SiO <sub>2</sub> )	1 - 4%
	- zeolite (as NaAlSiO <sub>4</sub> )	24 - 34%
	- sodium sulfate (as Na <sub>2</sub> SO <sub>4</sub> )	4 - 10%
10	- sodium citrate/citric acid (as C <sub>6</sub> H <sub>5</sub> Na <sub>3</sub> O <sub>7</sub> /C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> )	0 - 15 %
	- carboxymethylcellulose	0 - 2%
	- polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG)	1 - 6%
15	- enzymes	0 - 5%
	- minor ingredients (e.g. suds suppressors, perfume)	0 - 5%

3) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

20	- linear alkylbenzenesulfonate (calculated as acid)	5 - 9%
	- alcohol ethoxylate (e.g. C <sub>12-15</sub> alcohol, 7 EO)	7 - 14%
25	- soap as fatty acid (e.g. C <sub>16-22</sub> )	1 - 3%
	- sodium carbonate (as Na <sub>2</sub> CO <sub>3</sub> )	10 - 17%
	- soluble silicate (as Na <sub>2</sub> O, 2SiO <sub>2</sub> )	3 - 9%
	- zeolite (as NaAlSiO <sub>4</sub> )	23 - 33%
	- sodium sulfate (as Na <sub>2</sub> SO <sub>4</sub> )	0 - 4%
30	- sodium perborate (as NaBO <sub>3</sub> ·H <sub>2</sub> O)	8 - 16%
	- TAED	2 - 8%
	- phosphonate (e.g. EDTMPA)	0 - 1%

- carboxymethylcellulose 0 - 2%
- polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG) 1 - 3%
- enzymes 0 - 5%
- 5 - minor ingredients (e.g. suds suppressors, perfume, optical brightener) 0 - 5%

4) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

- 10 - linear alkylbenzenesulfonate (calculated as acid) 8 - 12%
- alcohol ethoxylate (e.g. C<sub>12-15</sub> alcohol, 7 EO) 10 - 25%
- sodium carbonate (as Na<sub>2</sub>CO<sub>3</sub>) 14 - 22%
- soluble silicate (as Na<sub>2</sub>O, 2SiO<sub>2</sub>) 1 - 5%
- 15 - zeolite (as NaAlSiO<sub>4</sub>) 25 - 35%
- sodium sulfate (as Na<sub>2</sub>SO<sub>4</sub>) 0 - 10%
- carboxymethylcellulose 0 - 2%
- polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG) 1 - 3%
- 20 - enzymes 0 - 5%
- minor ingredients (e.g. suds suppressors, perfume) 0 - 5%

5) An aqueous liquid detergent composition comprising

- 25 - linear alkylbenzenesulfonate (calculated as acid) 15 - 21%
- alcohol ethoxylate (e.g. C<sub>12-15</sub> alcohol, 7 EO or C<sub>12-15</sub> alcohol, 5 EO) 12 - 18%
- soap as fatty acid (e.g. oleic acid) 3 - 13%
- 30 - alkenylsuccinic acid (C<sub>12-14</sub>) 0 - 13%
- aminoethanol 8 - 18%

- citric acid	2 - 8%
- phosphonate	0 - 3%
- polymers (e.g. PVP, PEG)	0 - 3%
- borate (as $B_4O_7$ )	0 - 2%
5 - ethanol	0 - 3%
- propylene glycol	8 - 14%
- enzymes	0 - 5%
- minor ingredients (e.g. dispersants, suds suppressors, 10 perfume, optical brightener)	0 - 5%

6) An aqueous structured liquid detergent composition comprising

- linear alkylbenzenesulfonate (calculated as acid)	15 - 21%
15 - alcohol ethoxylate (e.g. $C_{12-15}$ alcohol, 7 EO or $C_{12-15}$ alcohol, 5 EO)	3 - 9%
- soap as fatty acid (e.g. oleic acid)	3 - 10%
- zeolite (as $NaAlSiO_4$ )	14 - 22%
20 - potassium citrate	9 - 18%
- borate (as $B_4O_7$ )	0 - 2%
- carboxymethylcellulose	0 - 2%
- polymers (e.g. PEG, PVP)	0 - 3%
- anchoring polymers as 25 e.g. lauryl methacrylate/acrylic acid copolymer; molar ratio 25:1; MW 3800	0 - 3%
- glycerol	0 - 5%
- enzymes	0 - 5%
- minor ingredients 30 (e.g. dispersants, suds suppressors, perfume, optical brighteners)	0 - 5%

7) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

- fatty alcohol sulfate	5 - 10%
- ethoxylated fatty acid monoethanolamide	3 - 9%
5 - soap as fatty acid	0 - 3%
- sodium carbonate (as $\text{Na}_2\text{CO}_3$ )	5 - 10%
- soluble silicate (as $\text{Na}_2\text{O}, 2\text{SiO}_2$ )	1 - 4%
- zeolite (as $\text{NaAlSiO}_4$ )	20 - 40%
- sodium sulfate (as $\text{Na}_2\text{SO}_4$ )	2 - 8%
10 - sodium perborate (as $\text{NaBO}_3 \cdot \text{H}_2\text{O}$ )	12 - 18%
- TAED	2 - 7%
- polymers (e.g. maleic/acrylic acid copolymer, PEG)	1 - 5%
- enzymes	0 - 5%
15 - minor ingredients (e.g. optical brightener, suds suppressors, perfume)	0 - 5%

8) A detergent composition formulated as a granulate comprising

- linear alkylbenzenesulfonate (calculated as acid)	8 - 14%
20 - ethoxylated fatty acid monoethanolamide	5 - 11%
- soap as fatty acid	0 - 3%
- sodium carbonate (as $\text{Na}_2\text{CO}_3$ )	4 - 10%
- soluble silicate (as $\text{Na}_2\text{O}, 2\text{SiO}_2$ )	1 - 4%
- zeolite (as $\text{NaAlSiO}_4$ )	30 - 50%
25 - sodium sulfate (as $\text{Na}_2\text{SO}_4$ )	3 - 11%
- sodium citrate (as $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7$ )	5 - 12%
- polymers (e.g. PVP, maleic/acrylic acid copolymer, PEG)	1 - 5%
- enzymes	0 - 5%
30 - minor ingredients (e.g. suds suppressors,	

perfume) 0 - 5%

- 9) A detergent composition formulated as a granulate comprising
- linear alkylbenzenesulfonate (calculated as acid) 6 - 12%
  - 5 - nonionic surfactant, 1 - 4%
  - soap as fatty acid 2 - 6%
  - sodium carbonate (as  $\text{Na}_2\text{CO}_3$ ) 14 - 22%
  - zeolite (as  $\text{NaAlSiO}_4$ ) 18 - 32%
  - sodium sulfate (as  $\text{Na}_2\text{SO}_4$ ) 5 - 20%
  - 10 - sodium citrate (as  $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7$ ) 3 - 8%
  - sodium perborate (as  $\text{NaBO}_3 \cdot \text{H}_2\text{O}$ ) 4 - 9%
  - bleach activator (e.g. NOBS or TAED) 1 - 5%
  - carboxymethylcellulose 0 - 2%
  - polymers (e.g. polycarboxylate or PEG) 1 - 5%
  - 15 - enzymes 0 - 5%
  - minor ingredients (e.g. optical brightener, perfume) 0 - 5%
- 10) An aqueous liquid detergent composition comprising
- 20 - linear alkylbenzenesulfonate (calculated as acid) 15 - 23%
  - alcohol ethoxysulfate (e.g.  $\text{C}_{12-15}$  alcohol, 2-3 EO) 8 - 15%
  - alcohol ethoxylate (e.g.  $\text{C}_{12-15}$  alcohol, 7 EO or  $\text{C}_{12-15}$  alcohol, 5 EO) 3 - 9%
  - 25 - soap as fatty acid (e.g. lauric acid) 0 - 3%
  - aminoethanol 1 - 5%
  - sodium citrate 5 - 10%
  - hydrotrope (e.g. sodium toluenesulfonate) 2 - 6%

- borate (as  $B_4O_7$ ) 0 - 2%
- carboxymethylcellulose 0 - 1%
- ethanol 1 - 3%
- propylene glycol 2 - 5%
- 5 - enzymes 0 - 5%
- minor ingredients (e.g. polymers, dispersants,  
perfume, optical brighteners) 0 - 5%

11) An aqueous liquid detergent composition comprising

- linear alkylbenzenesulfonate  
10 (calculated as acid) 20 - 32%
- alcohol ethoxylate  
(e.g.  $C_{12-15}$  alcohol, 7 EO  
or  $C_{12-15}$  alcohol, 5 EO) 6 - 12%
- aminoethanol 2 - 6%
- 15 - citric acid 8 - 14%
- borate (as  $B_4O_7$ ) 1 - 3%
- polymer (e.g. maleic/acrylic acid copolymer,  
anchoring polymers as e.g.  
lauryl methacrylate/acrylic acid  
20 copolymer and CMC) 0 - 3%
- glycerol 3 - 8%
- enzymes 0 - 5%
- minor ingredients (e.g. hydrotropes,  
dispersants, perfume, optical brighteners) 0 - 5%

25 12) A detergent composition formulated as a granulate having a  
bulk density of at least 600 g/l comprising

- anionic surfactant (linear  
alkylbenzenesulfonate, alkyl sulfate, alpha-  
olefinsulfonate, alpha-sulfo fatty acid  
30 methyl esters, alkanesulfonates, soap) 25 - 40%
- nonionic surfactant  
(e.g. alcohol ethoxylate) 1 - 10%
- sodium carbonate (as  $Na_2CO_3$ ) 8 - 25%



- soluble silicates (as $\text{Na}_2\text{O}$ , $2\text{SiO}_2$ )	5 - 15%
- sodium sulfate (as $\text{Na}_2\text{SO}_4$ )	0 - 5%
- zeolite (as $\text{NaAlSiO}_4$ )	15 - 28%
- sodium perborate (as $\text{NaBO}_3 \cdot 4\text{H}_2\text{O}$ )	0 - 20%
5 - bleach activator (TAED or NOBS)	0 - 5%
- enzymes	0 - 5%
- minor ingredients (e.g. perfume, optical brighteners)	0 - 3%

13) Detergent formulations as described in 1) - 12) where the  
10 content of linear alkylbenzenesulfonate - or a part of it - is  
substituted by alkyl sulfate ( $\text{C}_{12}\text{-C}_{18}$ ).

14) Detergent formulations as described in 1) - 13) which  
contain a stabilized or encapsulated peracid either as an  
additional component or as a substitute for already specified  
15 bleach systems.

15) Detergent compositions as described in 3), 7), 9) and 12)  
where the content of perborate is substituted with percar-  
bonate.

16) Detergent composition formulated as a nonaqueous detergent  
20 liquid comprising a liquid nonionic surfactant as e.g. linear  
alkoxylated primary alcohol, a builder system (e.g. phosphate),  
enzyme and alkali. The detergent may also comprise anionic  
surfactant and/or a bleach system.

The following examples further illustrate the present  
25 invention, and they are not intended to be in any way limiting  
to the scope of the invention as claimed.

#### EXAMPLE 1

### Bleaching of Direct Blue 1 with Bilirubin Oxidase and 10-Methylphenothiazine

The bleaching of Direct Blue 1 (DB1) by bilirubin oxidase obtained from Sigma with and without 10-methyl-phenothiazine (MPT) is presented in the attached Fig. 2.

The following conditions were used:

	Final concentrations
Buffer	50 mM phosphate, pH 7
MPT	0, 2.5, 5, or 10 $\mu$ M
10 DB1	$\approx \text{Abs}_{605\text{nm}} = 0.6$
Bilirubin oxidase	0.027 Sigma units/ml

The experiment was started by the addition of enzyme, and the absorbance at 605 nm was monitored for 5 minutes. The temperature was 25°C.

15 No attempt to maintain a constant concentration of dissolved oxygen was made.

As evidenced by Fig. 2, bleaching is enhanced by increasing concentrations of the enhancer of the invention.

### **EXAMPLE 2**

#### 20 Bleaching of Direct Blue 1

**Bilirubin oxidase:** Myrothecium verrucaria bilirubin oxidase was purchased from Sigma (B-0390). A stock solution was made containing 27 Sigma units/ml, which was stored in the freezer until use.

25 **Laccase obtained from Trametes villosa:** 800 ml culture broth of Trametes villosa, CBS 678.70, was filtered with filter aid to give a clear filtrate, which was concentrated and washed by ultrafiltration on a membrane with a cut-off of 6-8 kDa. One ml samples of concentrated preparation was applied onto a Q-  
30 Sepharose HP column (Pharmacia, Sweden) equilibrated with 0.1 M fosfate pH 7, and the laccase was eluted with a flat NaCl

gradient around 0.25 M. Fractions with laccase activity from 10 runs were pooled and concentrated by ultrafiltration to an activity of 500 LACU/ml.

**Enhancers:** Chemicals were obtained from Sigma-Aldrich, Janssen  
5 Chimica, Kodak, Tokyo Kasai Organic Chemicals or Daiichi Pure  
Chemicals Co. N-methylated derivatives of phenothiazine and  
phenoxazine were made by methylation with methyl iodide as  
described by Cornel Bodea and Ioan Silberg in "Recent Advances  
10 in the Chemistry of Phenothiazines" (Advances in heterocyclic  
chemistry, 1968, Vol. 9, pp. 321-460); B. Cardillo & G. Casnati  
in Tetrahedron, 1967, Vol. 23, p. 3771. Phenothiazine and  
phenoxazine propionic acids may be prepared as described in J.  
Org. Chem. 15, 1950, pp. 1125-1130. Hydroxyethyl and hydroxy-  
propyl derivatives of phenothiazine and phenoxazine may be  
15 prepared as described by G. Cauquil in Bulletin de la Society  
Chemique de France, 1960, p.1049.

Due to low solubility some of the enhancers were dissolved in a small volume of ethanol before dilution in water.

The following conditions were used:

20	Final concentration	
400 $\mu$ l 50 mM Britton-Robinson buffer,	20	mM
pH 5.5 and pH 7.0 respectively,		
200 $\mu$ l DB1 ~ 3.0 Abs. Units (610 nm)	0.6	(A <sub>610nm</sub> )
200 $\mu$ l 50 $\mu$ M enhancer	10	$\mu$ M
25	200 $\mu$ l Enzyme	

Reagents were mixed in a 1 cm thermostated cuvette at 30°C and the bleaching was started by addition of enzyme.

The bleaching was detected spectrophotometrically at 610 nm, which is the absorption peak of DB1. After 5 sec.  
30 bleaching was followed for 4 minutes.

From the results presented in Table 1-2, below, it appears that adding enhancers of the invention a much faster bleaching of the dye can be obtained compared to the experiment without enhancer. Enzyme dosages given are in the final  
35 incubation mixture.

Table 1

Bleaching of Direct Blue 1 with Trametes villosa laccase, obtained as described above, at pH 5.5 (0.1 LACU/ml) and pH 7.0 (1 LACU/ml). \_

5 Enhancer DB1 bleaching in 4 minutes  
(-ΔmAbs/4 min)  
pH 5.5 pH 7.0

	No enhancer	0	0
10	10-Phenothiazine-propionic acid	387	182
	10-Methyl-phenothiazine	222	22
15	4'-Hydroxy-4-biphenyl-carboxylic acid	46	34
	4,4'-Diaminostilbene-2,2'-disulfonic acid	3.5	6
20	6-Hydroxy-2-naphthoic acid	12	20
	10-Methylphenoxazine	364	296
25	10-Phenoxazinepropionic acid	364	268
	10-Ethyl-4-phenothiazinepropionic acid	411	175
30	10-(2-Hydroxyethyl)-phenothiazine	373	184
	10-(2-Hydroxyethyl)-phenoxazine	429	192
35	10-(3-Hydroxypropyl)-phenothiazine	428	219
	4,4'-Dimethoxy-N-		

35

methy1-diphenylamine	210	113
Vanillin azine	118	5

Table 2

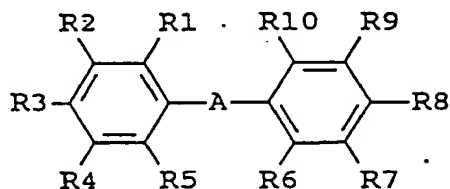
Bleaching of Direct Blue 1 with Myrothecium verrucaria bilirubin oxidase, obtained as described above, at pH 5.5 (0.00675 Sigma units/ml) and pH 7.0 (0.0675 Sigma units/ml).

Enhancer	DB1 bleaching in 4 minutes (-ΔmAbs/4 min)	
	pH 5.5	pH 7.0
No enhancer	68	207
10-Phenothiazine-propionic acid	95	244
15 10-Methylphenothiazine	76	250
10-Methylphenoxazine	143	374
10-Phenoxazinepropionic acid	96	347
20 10-Ethyl-4-phenothiazinepropionic acid	103	293

## CLAIMS

1. A method of oxidizing a substrate with an enzyme selected from the group consisting of laccase, chatechol oxidase, monophenol monooxygenase and bilirubin oxidase, characterized by the presence of an enhancing agent being an organic chemical compound consisting of at least two aromatic rings, of which aromatic rings at least one is substituted with one or more of the following atoms: nitrogen, oxygen, and sulfur; and which aromatic rings may furthermore be fused rings.

2. A method according to claim 1 in which the enhancing agent is described by the general formula I:



in which general formula A represents a single bond, or one of the following groups:  $(-CR^{11}=CR^{15}-)_n$ ,  $(-CR^{11}=N-N=CR^{15}-)$ ,  $(-NR^{11}-)$ ,  $(-CR^{12}=N-)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{15}-)$ ,  $(-NR^{11}-CR^{12}=N-)_n$ ,  $(-CR^{12}=N-NR^{11}-)$ ,  $(-NR^{11}-CR^{12}=CR^{13}-)$ ,  $(-N=N-)$ , in which groups n represents an integer of from 1 to 6; or A represents carbon, carbonyl, nitrogen, sulfur, oxygen, selenium, or phosphor, which carbon, phosphor and nitrogen may be unsubstituted or substituted with a substituent group  $R^{11}$ ;

and in which general formula the substituent groups  $R^1$ - $R^{13}$  and  $R^{15}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, sulfandiyl, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and

amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{14}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ;

which substituent group  $R^{14}$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, aminoalkyl, piperidino, piperaziny, pyrrolidino,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

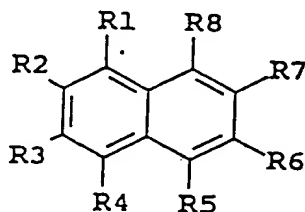
or in which general formula  $R^5$  and  $R^6$  may together form a group -B-, in which B represents a single bond, the group  $(-CH=CH-)_n$ , or the group  $(-CH=N-)_n$ , in which groups n represents an integer of from 1 to 6; or B represents carbon, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group  $R^{14}$  as defined above;

or in which general formula two of the substituent groups  $R^1$ - $R^{10}$  may together form a group -C-, in which C represents any of the following groups:  $(-CHR^{11}-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$ , in which groups n represents an integer of from 2 to

4, and in which groups  $R^{11}$  is a substituent group as defined above;

or in which general formula  $R^5$  and  $R^{12}$ , and/or  $R^6$  and  $R^{13}$ , when  $n$  in the above formula being 1, may together form a group -D-, in which D represents the groups:  $(-CHR^{11}-)$ ,  $(-NR^{11}-)$ ,  $(-CR^{11}=CR^{15}-)$ ,  $(-CR^{11}=N-)$ ,  $(-N=CR^{11}-)$ ,  $(-O-)$ ,  $(>C=O)$  or  $(-S-)$ , and in which groups  $R^{11}$  and  $R^{15}$  are substituent groups as defined above.

3. A method according to claim 1, in which the enhancing agent is described by the general formula II:



in which the substituent groups  $R^1$ - $R^8$ , which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^9$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ;

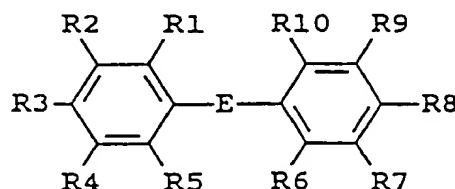
which substituent group  $R^9$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof,



sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups R<sup>1</sup>-R<sup>8</sup> may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub>, (-N=CR<sup>9</sup>-NR<sup>10</sup>-) or (-N=N-CR<sup>9</sup>-), in which groups n represents an integer of from 1 to 3, R<sup>9</sup> is a substituent group as defined above and R<sup>10</sup> is defined as R<sup>9</sup>.

4. A method according to either of claims 1-2, in which the enhancing agent is described by the following formula:



in which formula E represents a single bond, a carbonyl group or one of the following groups: (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub> or (-NR<sup>11</sup>-), in which n represents an integer from 1 to 2. The substituents groups R<sup>1</sup>-R<sup>11</sup> may be identical or different, independently being one of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, car-

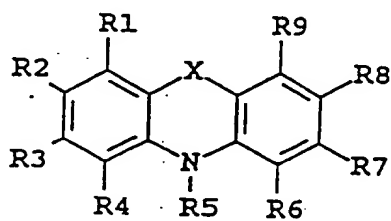
bamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups  
5 may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>14</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>14</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or un-  
10 saturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>14</sup>;

which substituent group R<sup>14</sup> represents any of the following radicals: hydroxy, formyl, carboxy and esters and  
15 salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof. nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-  
20 alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be  
25 saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

30 or in which specific formula two of the substituent groups R<sup>5</sup> and R<sup>6</sup> may together form a group -B-, in which B represents the groups: (-CH=N-)<sub>n</sub>, (-CH=CH-) or (-CHR<sup>14</sup>-) in which groups n represents an integer of from 1 to 2 and R<sup>14</sup> is a substituent group as defined above.

5. A method according to claim 4, in which the enhancing agent is 4-amino-4'-methoxystilbene, 4,4'-diaminostilbene-2,2'-disulfonic acid, iminostilbene, 4,4'-dihydroxybenzophenone, N-benzylidene-4-biphenylamine, 4,4'-diaminodiphenylamine, 4,4'-dimethoxy-N-methyl-diphenylamine, 2,7-diaminofluorene or triphenylamine.

6. A method according to either of claims 1-2, in which the enhancing agent is described by the following formula:



10 in which formula X represents one of the following groups: (-O-), (-S-), (-NR<sup>15</sup>-), (-CHR<sup>15</sup>-), (>C=O), (-CH=CH-), (-CH=N-), (-N=N-) and the substituent groups R<sup>1</sup>-R<sup>9</sup> and R<sup>15</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and  
 15 esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or  
 20 substituted once or twice with a substituent group R<sup>10</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>10</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups

may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{10}$ ;

which substituent group  $R^{10}$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

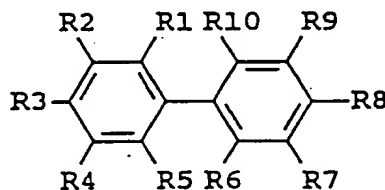
or in which general formula two of the substituent groups  $R^1$ - $R^8$  may together form a group -B-, in which B represents any of the following the groups:  $(-CHR^{10}-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$  or  $(-N=CR^{10}-NR^{11}-)$ , in which groups n represents an integer of from 1 to 3,  $R^{10}$  is a substituent group as defined above and  $R^{11}$  is defined as  $R^{10}$ .

7. A method according to claim 6, in which X in the formula is  $(-O-)$  or  $(-S-)$  and  $R^5$  in the formula is an alkyl group which may be further substituted.

8. A method according to either of claims 6-7, in which the enhancing agent is 10-methylphenothiazine, 10-phenothiazine-propionic acid, N-hydroxysuccinimide-10-phenothiazine-propionate, 10-ethyl-4-phenothiazine-carboxylic acid,

10-ethylphenothiazine, 10-propylphenothiazine, 10-isopropylphenothiazine, methyl-10-phenothiazinepropionate, 10-phenylphenothiazine, 10-allylphenothiazine, 10-(3-(4-methyl-1-piperazinyl)propyl)phenothiazine, 10-(2-pyrrolidinoethyl)phenothiazine, 2-acetyl-10-methylphenothiazine, 4-carboxy-10-phenothiazine-propionic acid, 10-methylphenoxazine, 10-ethylphenoxazine, 10-phenoxazine-propionic acid, 4-carboxy-10-phenoxazine-propionic acid, 10-(2-hydroxyethyl)phenothiazine, 10-(2-hydroxyethyl)phenoxazine or 10-(3-hydroxypropyl)phenothiazine.

- 10 9. A method according to either of claims 1-2, in which the enhancing agent is a biphenyl derivative of the following formula:



in which the substituent groups  $R^1$ - $R^{10}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{11}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{11}$ ; and which  $C_1$ - $C_{14}$ -alkyl,

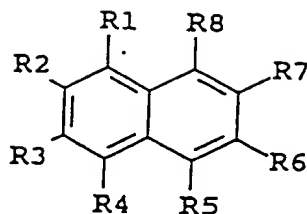
C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>11</sup>;

5           which substituent group R<sup>11</sup> represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy;  
10 which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof,  
15 carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters  
20 and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl.

10. A method according to claim 9, in which the enhancing agent is benzidine, 3,3'-dimethylbenzidine, 3,3'-dimethoxybenzidine, 3,3',5,5'-tetramethylbenzidine, 4'-hydroxy-  
25 4-biphenylcarboxylic acid, or 4,4'-dihydroxybiphenylene.

11. A method according to either of claims 1-2, in which the enhancing agent is 2-(p-aminophenyl)-6-methylbenzothiazole-7-sulfonic acid, N-(4-(dimethylamino)benzylidene)-p-anisidine, 3-methyl-2-benzothiazolinone(4-(dimethylamino)benzylidene)hydrazone or vanillin azine.  
30

12. A method according to either of claims 1 and 3, in which the enhancing agent is a naphthalene derivative of the following formula:



in which the substituent groups  $R^1$ - $R^8$ , which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^9$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ;

which substituent group  $R^9$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_3$ -alkyl,  $C_1$ - $C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters

and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups  $R^1$ - $R^8$  may together form a group -B-, in which B represents any of the following groups:  $(-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$ ,  $(-N=CR^9-NR^{10}-)$  or  $(-N=N-CR^9-)$ , in which groups n represents an integer of from 1 to 3,  $R^9$  is a substituent group as defined above and  $R^{10}$  is defined as  $R^9$ .

13. A method according to claim 12, in which the enhancing agent is 6-hydroxy-2-naphtoic acid, 7-methoxy-2-naphtol, 7-amino-2-naphthalene sulfonic acid, 5-amino-2-naphthalene sulfonic acid, 1,5-diaminonaphthalene, 7-hydroxy-1,2-naphthimidazole, 5-amino-2-naphthalenesulfonic acid, or 7-methoxy-2-naphtol.

15 14. A method according to any of claims 1-13, in which said method is a method for bleaching of dye in solutions.

15. A method according to any of claims 1-13, in which said method is a method for inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor.

16. A method according to any of claims 1-13, in which said method is a method for bleaching of lignin-containing material, in particular bleaching of pulp for paper production.

17. A method according to any of claims 1-13, in which said method is a method for enzymatic polymerization and/or modification of lignin or lignin containing material.

18. Use of the method according to claim 17 in the manufacture of wood composites such as chipboards, fibre



boards, and particle boards, or in the manufacture of laminated wood products such as laminated beams and plywood.

19. A method according to any of claims 1-13, in which said method is a method for treatment of waste water, in particular waste water from the pharmaceutical or chemical industry, e.g. waste water from dye manufacturing, from textile industry, or from pulp manufacturing.

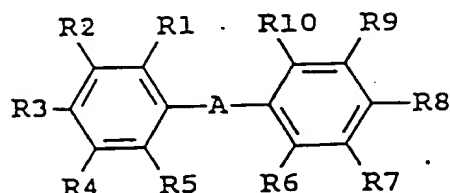
20. A method according to any of claims 14-19, in which the enhancing agent is added at the beginning of, or during the process.

21. A method according to any of claims 14-20, in which the amount of enhancing agent is in the range of from 0.01-500  $\mu\text{M}$ , more preferred 0.1-250  $\mu\text{M}$ , most preferred 0.5-100  $\mu\text{M}$ .

22. A method according to any of claims 14-21, in which the laccase enzyme is derived from Trametes, e.g. T. versicolor or T. villosa, Collybia, Fomes, Lentinus, Pleurotus, Rhizoctonia, e.g. R. solani, Aspergillus, Neurospora, Podospora, Phlebia, Myceliophthora, e.g. M. thermophila, Botrytis, or the bilirubin oxidase is derived from Myrothecium, e.g. M. verrucaria.

23. A detergent additive capable of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the detergent additive comprising an enzyme exhibiting laccase activity, characterized by the presence of an enhancing agent being an organic chemical compound consisting of at least two aromatic rings, of which aromatic rings at least one is substituted with one or more of the following atoms: nitrogen, oxygen, and sulfur; and which aromatic rings may furthermore be fused rings.

24. A detergent additive according to claim 23, in which the enhancing agent is described by the general formula I:



in which general formula A represents a single bond, or one of the following groups:  $(-CR^{11}=CR^{15}-)_n$ ,  $(-CR^{11}=N-N=CR^{15}-)$ ,  $(-NR^{11}-)$ ,  $(-CR^{12}=N-)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{15}-)$ ,  $(-NR^{11}-CR^{12}=N-)_n$ ,  $(-CR^{12}=N-NR^{11}-)$ ,  $(-NR^{11}-CR^{12}=CR^{13}-)$ ,  $(-N=N-)$ , in which groups n represents an integer of from 1 to 6; or A represents carbon, carbonyl, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group  $R^{11}$ ;

and in which general formula the substituent groups  $R^1$ - $R^{13}$  and  $R^{15}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{14}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ;

which substituent group  $R^{14}$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and

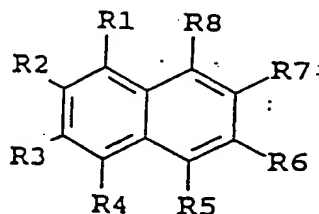
salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, aminoalkyl, piperidino, piperaziny, pyrrolidino, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which  
5 carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof,  
10 carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters  
15 and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula R<sup>5</sup> and R<sup>6</sup> may together form a group -B-, in which B represents a single bond, the group (-CH=CH-)<sub>n</sub>, or the group (-CH=N-)<sub>n</sub>, in which groups n  
20 represents an integer of from 1 to 6; or B represents carbon, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group R<sup>14</sup> as defined above;

or in which general formula two of the substituent  
25 groups R<sup>1</sup>-R<sup>10</sup> may together form a group -C-, in which C represents any of the following groups: (-CHR<sup>11</sup>-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub>, in which groups n represents an integer of from 2 to 4, and in which groups R<sup>11</sup> is a substituent group as defined above;

30 or in which general formula R<sup>5</sup> and R<sup>12</sup>, and/or R<sup>6</sup> and R<sup>13</sup>, when n in the above formula being 1, may together form a group -D-, in which D represents the groups: (-CHR<sup>11</sup>-), (-NR<sup>11</sup>-), (-CR<sup>11</sup>=CR<sup>15</sup>-), (-CR<sup>11</sup>=N-), (-N=CR<sup>11</sup>-), (-O-), (>C=O) or (-S-), and in which groups R<sup>11</sup> and R<sup>15</sup> are substituent groups as defined  
35 above.

25. A detergent additive according to claim 23, in which the enhancing agent is described by the general formula II:



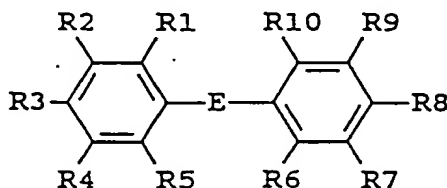
in which the substituent groups  $R^1$ - $R^8$ , which may be  
 5 identical or different, independently represents any of the  
 following radicals: hydrogen, hydroxy, formyl, carboxy and  
 esters and salts hereof, carbamoyl, sulfo and esters and salts  
 hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters  
 and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -  
 10 alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl,  
 sulfamoyl, and amino groups may furthermore be unsubstituted or  
 substituted once or twice with a substituent group  $R^9$ ; and which  
 phenyl may furthermore be unsubstituted or substituted with one  
 or more substituent groups  $R^9$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -  
 15 alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be  
 saturated or unsaturated, branched or unbranched, and may  
 furthermore be unsubstituted or substituted with one or more  
 substituent groups  $R^9$ ;

which substituent group  $R^9$  represents any of the  
 20 following radicals: hydroxy, formyl, carboxy and esters and  
 salts hereof, carbamoyl, sulfo and esters and salts hereof,  
 sulfamoyl, phospho, phosphono, phosphonooxy and esters and  
 salts thereof, nitro, amino, phenyl,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy;  
 which carbamoyl, sulfamoyl, and amino groups may furthermore be  
 25 unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_3$ -

alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups R<sup>1</sup>-R<sup>8</sup> may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub>, (-N=CR<sup>9</sup>-NR<sup>10</sup>-) or (-N=N-CR<sup>9</sup>-), in which groups n represents an integer of from 1 to 3, R<sup>9</sup> is a substituent group as defined above and R<sup>10</sup> is defined as R<sup>9</sup>.

26. A detergent additive according to either of claims 23-24, in which the enhancing agent is described by the following formula:



in which formula E represents a single bond, a carbonyl group or one of the following groups: (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub> or (-NR<sup>11</sup>-), in which n represents an integer from 1 to 2. The substituents groups R<sup>1</sup>-R<sup>11</sup> may be identical or different, independently being one of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl,

aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>14</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>14</sup>; and which C<sub>1</sub>-C<sub>4</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>14</sup>;

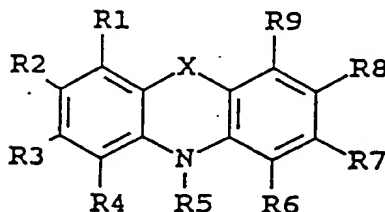
10           which substituent group R<sup>14</sup> represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; 15 which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, 20 carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters 25 and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

          or in which specific formula two of the substituent groups R<sup>5</sup> and R<sup>6</sup> may together form a group -B-, in which B represents the groups: (-CH=N-)<sub>n</sub>, (-CH=CH-) or (-CHR<sup>14</sup>-) in which 30 groups n represents an integer of from 1 to 2 and R<sup>14</sup> is a substituent group as defined above.

27. A detergent additive according to claim 26, in which the enhancing agent is 4-amino-4'-methoxystilbene, 4,4'-diaminostilbene-2,2'-disulfonic acid, iminostilbene, 4,4'- 35 dihydroxybenzophenone, N-benzylidene-4-biphenylamine, 4,4'-

diaminodiphenylamine, 4,4'-dimethoxy-N-methyl-diphenylamine, 2,7-diaminofluorene or triphenylamine.

28. A detergent additive according to either of claims 23-24, in which the enhancing agent is described by the following formula:



in which formula X represents one of the following groups: (-O-), (-S-), (-NR<sup>15</sup>-), (-CHR<sup>15</sup>-), (>C=O), (-CH=CH-), (-CH=N-) and the substituent groups R<sup>1</sup>-R<sup>9</sup> and R<sup>15</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>10</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>10</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>10</sup>;

which substituent group  $R^{10}$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, aminoalkyl, piperidino, piperaziny, pyrrolidino,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups  $R^1$ - $R^8$  may together form a group -B-, in which B represents any of the following the groups:  $(-CHR^{10}-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$  or  $(-N=CR^{10}-NR^{11}-)$ , in which groups n represents an integer of from 1 to 3,  $R^{10}$  is a substituent group as defined above and  $R^{11}$  is defined as  $R^{10}$ .

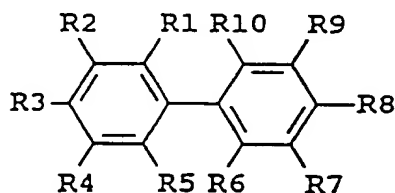
29. A detergent additive according to claim 28, in which X in the formula is  $(-O-)$  or  $(-S-)$  and  $R^5$  in the formula is an alkyl group which may be further substituted.

30. A detergent additive according to either of claims 28-29, in which the enhancing agent is 10-methylphenothiazine, 10-phenothiazine-propionic acid, N-hydroxysuccinimide-10-phenothiazine-propionate, 10-ethyl-4-phenothiazine-carboxylic acid, 10-ethylphenothiazine, 10-propylphenothiazine, 10-isopropylphenothiazine, methyl-10-phenothiazinepropionate, 10-phenylphenothiazine, 10-allylphenothiazine, 10-(3-(4-methyl-



1-piperazinyl)propyl)phenothiazine, 10-(2-pyrrolidinoethyl)-phenothiazine, 2-acetyl-10-methylphenothiazine, 10-methylphenoxazine, 10-(2-hydroxyethyl)phenothiazine, 10-(2-hydroxyethyl)phenoxazine or 10-(3-hydroxypropyl)phenothiazine.

- 5 31. A detergent additive according to either of claims 23-24, in which the enhancing agent is a biphenyl derivative of the following formula:



in which the substituent groups  $R^1$ - $R^{10}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{11}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{11}$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{11}$ ;

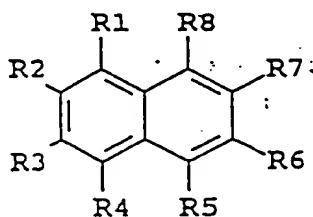
which substituent group  $R^{11}$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be

unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, 5 carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters 10 and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl.

32. A detergent additive according to claim 31, in which the enhancing agent is benzidine, 3,3'-dimethylbenzidine, 3,3'-dimethoxybenzidine, 3,3',5,5'-tetramethylbenzidine, 4'- 15 hydroxy-4-biphenylcarboxylic acid, or 4,4'-dihydroxybiphenylene.

33. A detergent additive according to either of claims 23-24, in which the enhancing agent is 2-(p-amino-phenyl)-6-methylbenzothiazole-7-sulfonic acid, N-(4-(dimethyl- 20 amino)benzylidene)-p-anisidine, 3-methyl-2-benzothiazolinone(4-(dimethylamino)benzylidene)hydrazone or vanillin azine.

34. A detergent additive according to either of claims 23 and 25, in which the enhancing agent is a naphthalene derivative of the following formula:



25 in which the substituent groups R<sup>1</sup>-R<sup>8</sup>, which may be identical or different, independently represents any of the

following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>9</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>;

which substituent group R<sup>9</sup> represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

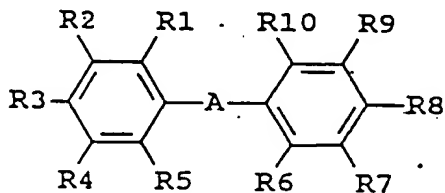
or in which general formula two of the substituent groups R<sup>1</sup>-R<sup>8</sup> may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub>, (-N=CR<sup>9</sup>-NR<sup>10</sup>-) or (-N=N-CR<sup>9</sup>-), in which groups n represents an integer of from 1 to 3, R<sup>9</sup> is a substituent group as defined above and R<sup>10</sup> is defined as R<sup>9</sup>.

35. A detergent additive according to claim 34, in which the enhancing agent is 6-hydroxy-2-naphtoic acid, 7-methoxy-2-naphtol, 7-amino-2-naphthalene sulfonic acid, 5-amino-2-naphthalene sulfonic acid, 1,5-diaminonaphthalene, 7-hydroxy-1,2-naphthimidazole, 5-amino-2-naphthalenesulfonic acid, or 7-methoxy-2-naphtol.

36. A detergent additive according to any of claims 23-35, provided in the form of a granulate, preferably a non-dusting granulate, a liquid, in particular a stabilized liquid, a slurry, or a protected enzyme.

37. A detergent composition capable of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the detergent composition comprising an enzyme exhibiting laccase activity, characterized by the presence of an enhancing agent being an organic chemical compound consisting of at least two aromatic rings, of which aromatic rings at least one is substituted with one or more of the following atoms: nitrogen, oxygen, and sulfur; and which aromatic rings may furthermore be fused rings.

38. A detergent composition according to claim 37, in which the enhancing agent is described by the general formula I:



in which general formula A represents a single bond, or one of the following groups:  $(-CR^{11}=CR^{15}-)_n$ ,  $(-CR^{11}=N-N=CR^{15}-)$ ,  $(-NR^{11}-)$ ,  $(-CR^{12}=N-)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{15}-)$ ,  $(-NR^{11}-CR^{12}=N-)_n$ ,  $(-CR^{12}=N-NR^{11}-)$ ,  $(-NR^{11}-CR^{12}=CR^{13}-)$ ,  $(-N=N-)$ , in which groups n

represents an integer of from 1 to 6; or A represents carbon, carbonyl, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group  $R^{11}$ ;

5 and in which general formula the substituent groups  $R^1$ - $R^{13}$  and  $R^{15}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho,  
10 phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{14}$ ; and which phenyl may furthermore  
15 be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent  
20 groups  $R^{14}$ ;

which substituent group  $R^{14}$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and  
25 salts thereof, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; and which phenyl may furthermore be  
30 substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may  
35 furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters

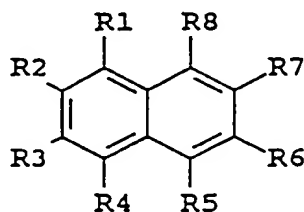
and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula  $R^5$  and  $R^6$  may together form a group -B-, in which B represents a single bond, the group  $(-CH=CH-)_n$ , or the group  $(-CH=N-)_n$ , in which groups n represents an integer of from 1 to 6; or B represents carbon, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group  $R^{14}$  as defined above;

or in which general formula two of the substituent groups  $R^1$ - $R^{10}$  may together form a group -C-, in which C represents any of the following groups:  $(-CHR^{11}-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$ , in which groups n represents an integer of from 2 to 4, and in which groups  $R^{11}$  is a substituent group as defined above;

or in which general formula  $R^5$  and  $R^{12}$ , and/or  $R^6$  and  $R^{13}$ , when n in the above formula being 1, may together form a group -D-, in which D represents the groups:  $(-CHR^{11}-)$ ,  $(-NR^{11}-)$ ,  $(-CR^{11}=CR^{15}-)$ ,  $(-CR^{11}=N-)$ ,  $(-N=CR^{11}-)$ ,  $(-O-)$ ,  $(>C=O)$  or  $(-S-)$ , and in which groups  $R^{11}$  and  $R^{15}$  are substituent groups as defined above.

39. A detergent composition according to claim 38, in which the enhancing agent is described by the general formula II:



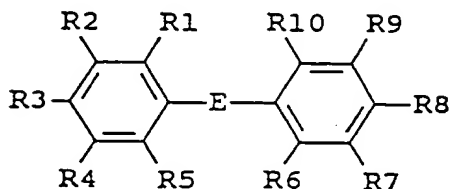
in which the substituent groups  $R^1$ - $R^8$ , which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and

esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, 5 sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>9</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be 10 saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>;

which substituent group R<sup>9</sup> represents any of the following radicals: hydroxy, formyl, carboxy and esters and 15 salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>- 20 alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be 25 saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

30 or in which general formula two of the substituent groups R<sup>1</sup>-R<sup>8</sup> may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub>, (-N=CR<sup>9</sup>-NR<sup>10</sup>-) or (-N=N-CR<sup>9</sup>-), in which groups n represents an integer of from 1 to 3, R<sup>9</sup> is a substituent group as 35 defined above and R<sup>10</sup> is defined as R<sup>9</sup>.

40. A detergent composition according to either of claims 37-38, in which the enhancing agent is described by the following formula:



in which formula E represents a single bond, a carbonyl group or one of the following groups:  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$  or  $(-NR^{11}-)$ , in which n represents an integer from 1 to 2. The substituents groups  $R^1-R^{11}$  may be identical or different, independently being one of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ -alkyl, aryl- $C_1-C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{14}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ -alkyl, and aryl- $C_1-C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ;

which substituent group  $R^{14}$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and

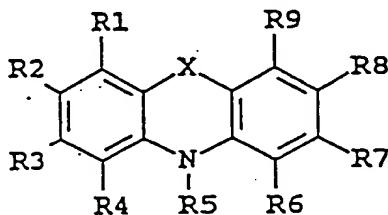


salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which specific formula two of the substituent groups R<sup>5</sup> and R<sup>6</sup> may together form a group -B-, in which B represents the groups: (-CH=N-)<sub>n</sub>, (-CH=CH-) or (-CHR<sup>14</sup>-) in which groups n represents an integer of from 1 to 2 and R<sup>14</sup> is a substituent group as defined above.

41. A detergent composition according to claim 40, in which the enhancing agent is 4-amino-4'-methoxystilbene, 4,4'-diaminostilbene-2,2'-disulfonic acid, iminostilbene, 4,4'-dihydroxybenzophenone, N-benzylidene-4-biphenylamine, 4,4'-diaminodiphenylamine, 4,4'-dimethoxy-N-methyldiphenylamine, 2,7-diaminofluorene or triphenylamine.

42. A detergent composition according to either of claims 37-38, in which the enhancing agent is described by the following formula:



in which formula X represents one of the following groups: (-O-), (-S-), (-NR<sup>15</sup>-), (-CHR<sup>15</sup>-), (>C=O), (-CH=CH-), (-CH=N-) and the substituent groups R<sup>1</sup>-R<sup>9</sup> and R<sup>15</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>10</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>10</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>10</sup>;

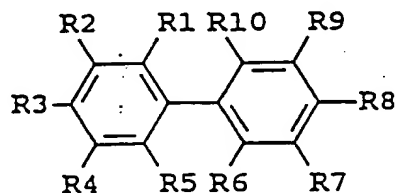
which substituent group R<sup>10</sup> represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups  $R^1$ - $R^8$  may together form a group -B-, in which B represents any of the following the groups:  $(-CHR^{10}-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$  or  $(-N=CR^{10}-NR^{11}-)$ , in which groups n represents an integer of from 1 to 3,  $R^{10}$  is a substituent group as defined above and  $R^{11}$  is defined as  $R^{10}$ .

43. A detergent composition according to claim 42, in which X in the formula is  $(-O-)$  or  $(-S-)$  and  $R^5$  in the formula is an alkyl group which may be further substituted.

10 44. A method according to either of claims 42-43, in which the enhancing agent is 10-methylphenothiazine, 10-phenothiazine-propionic acid, N-hydroxysuccinimide-10-phenothiazine-propionate, 10-ethyl-4-phenothiazine-carboxylic acid, 10-ethylphenothiazine, 10-propylphenothiazine, 10-isopropyl-  
15 phenothiazine, methyl-10-phenothiazinepropionate, 10-phenylphenothiazine, 10-allylphenothiazine, 10-(3-(4-methyl-1-piperazinyl)propyl)phenothiazine, 10-(2-pyrrolidinoethyl)-phenothiazine, 2-acetyl-10-methylphenothiazine, 10-methylphenoxazine, 10-(2-hydroxyethyl)phenothiazine, 10-(2-hydroxy-  
20 ethyl)phenoxazine or 10-(3-hydroxypropyl)phenothiazine.

45. A detergent composition according to either of 37-38, in which the enhancing agent is a biphenyl derivative of the following formula:



in which the substituent groups  $R^1$ - $R^{10}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{11}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{11}$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{11}$ ;

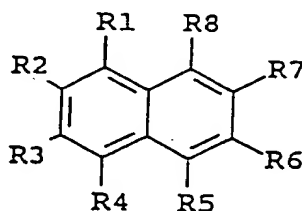
which substituent group  $R^{11}$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_3$ -alkyl,  $C_1$ - $C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl.

46. A detergent composition according to claim 45, in which the enhancing agent is benzidine, 3,3'-dimethylbenzidine, 3,3'-dimethoxybenzidine, 3,3',5,5'-tetramethyl-

benzidine, 4'-hydroxy-4-biphenylcarboxylic acid, or 4,4'-dihydroxybiphenylene.

47. A detergent composition according to either of claims 37-38, in which the enhancing agent is 2-(p-amino-5 phenyl)-6-methylbenzothiazole-7-sulfonic acid, N-(4-(dimethyl-amino)benzylidene)-p-anisidine, 3-methyl-2-benzothiazolinone(4-(dimethylamino)benzylidene)hydrazone or vanillin azine.

48. A detergent composition according to either of claims 37 and 39, in which the enhancing agent is a naphthalene  
10 derivative of the following formula:



in which the substituent groups R<sup>1</sup>-R<sup>8</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts  
15 hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>9</sup>; and which  
20 phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more  
25 substituent groups R<sup>9</sup>;

which substituent group R<sup>9</sup> represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof,

sulfamoyl, phospho, phosphono, phosphonoxy and esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

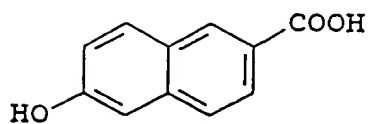
or in which general formula two of the substituent groups R<sup>1</sup>-R<sup>8</sup> may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub>, (-N=CR<sup>9</sup>-NR<sup>10</sup>-) or (-N=N-CR<sup>9</sup>-), in which groups n represents an integer of from 1 to 3, R<sup>9</sup> is a substituent group as defined above and R<sup>10</sup> is defined as R<sup>9</sup>.

49. A detergent composition according to claim 48, in which the enhancing agent is 6-hydroxy-2-naphtoic acid, 7-methoxy-2-naphtol, 7-amino-2-naphthalene sulfonic acid, 5-amino-2-naphthalene sulfonic acid, 1,5-diaminonaphthalene, 7-hydroxy-1,2-naphthimidazole, 5-amino-2-naphthalenesulfonic acid, or 7-methoxy-2-naphtol.

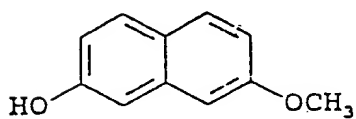
50. A detergent composition according to any of claims 37-49, which further comprises one or more other enzymes, in particular a protease, a lipase, an amylase, a cellulase, and/or an peroxidase.

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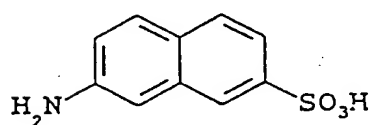
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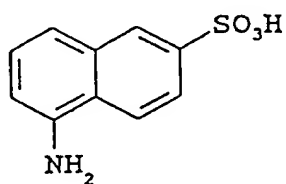
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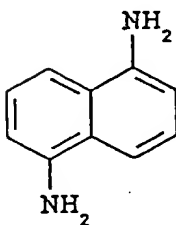
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IV



V



VI

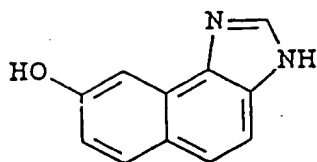


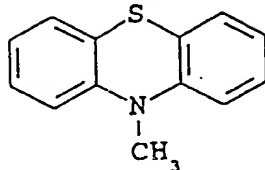
Fig. 1A



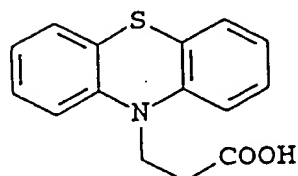


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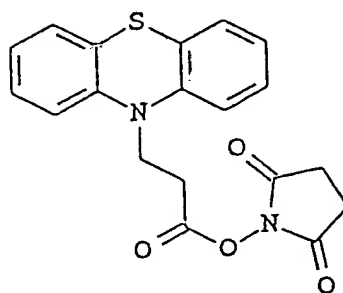
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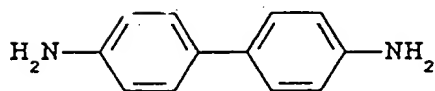
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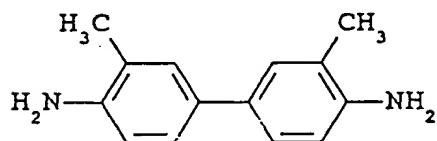
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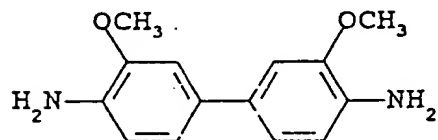
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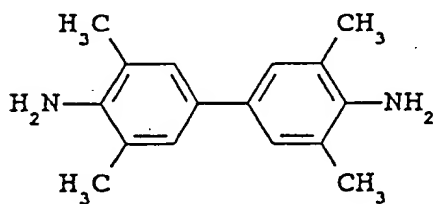
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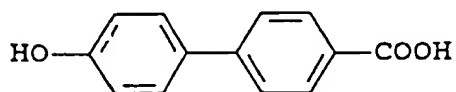
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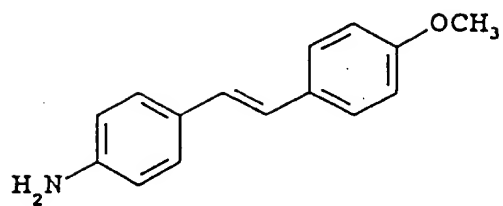
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**Fig. 1B**

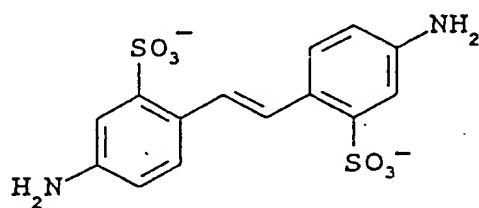


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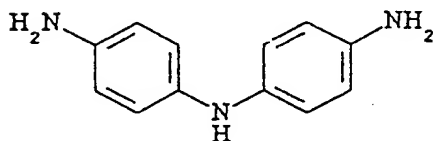
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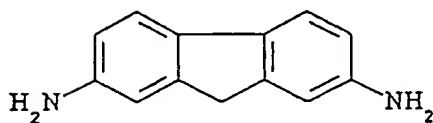
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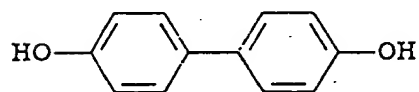
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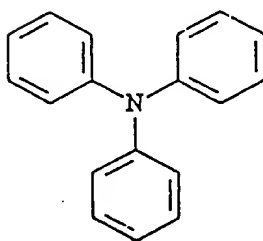
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XIX



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**Fig. 1C**



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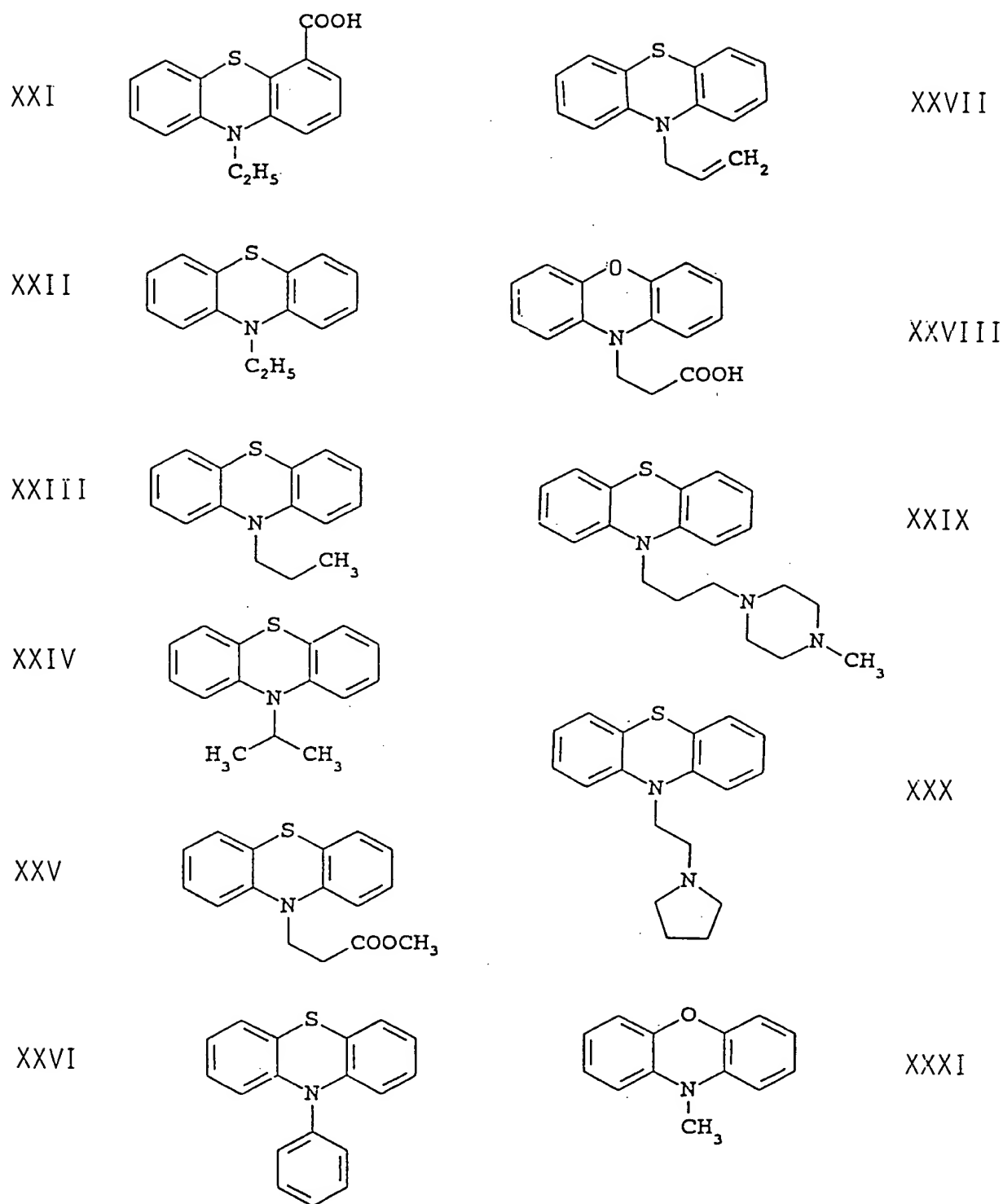
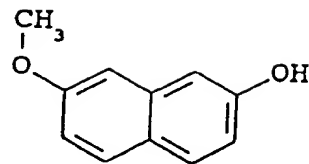
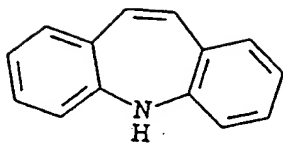


Fig. 1D



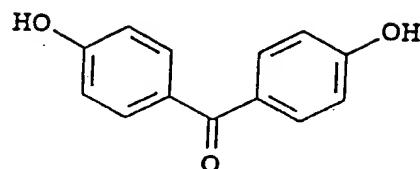
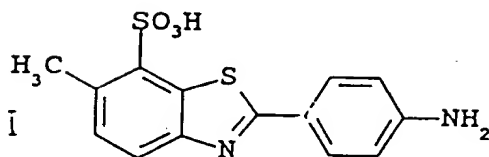
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XXXII



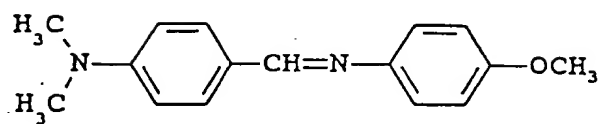
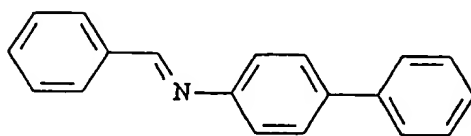
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XXXIII



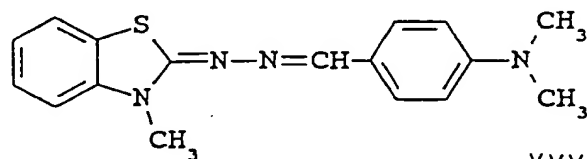
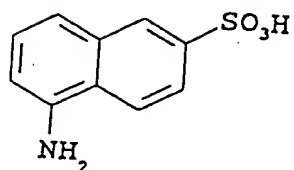
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XXXIV

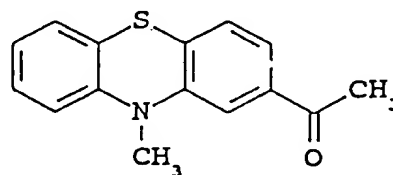


XXXVIII

XXXV



XXXIX



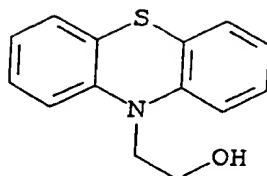
XL

Fig. 1E

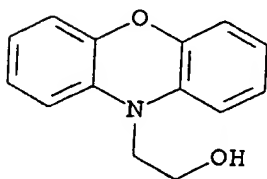




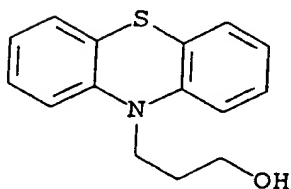
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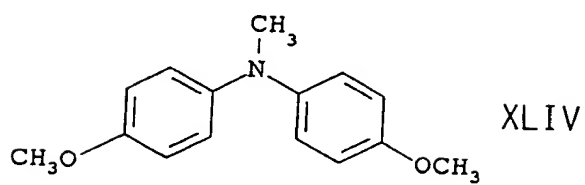
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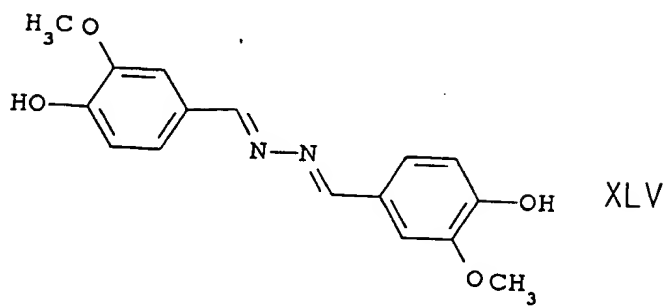
XLII



XLIII



XLIV



XLV

Fig. 1F



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A 605 nm

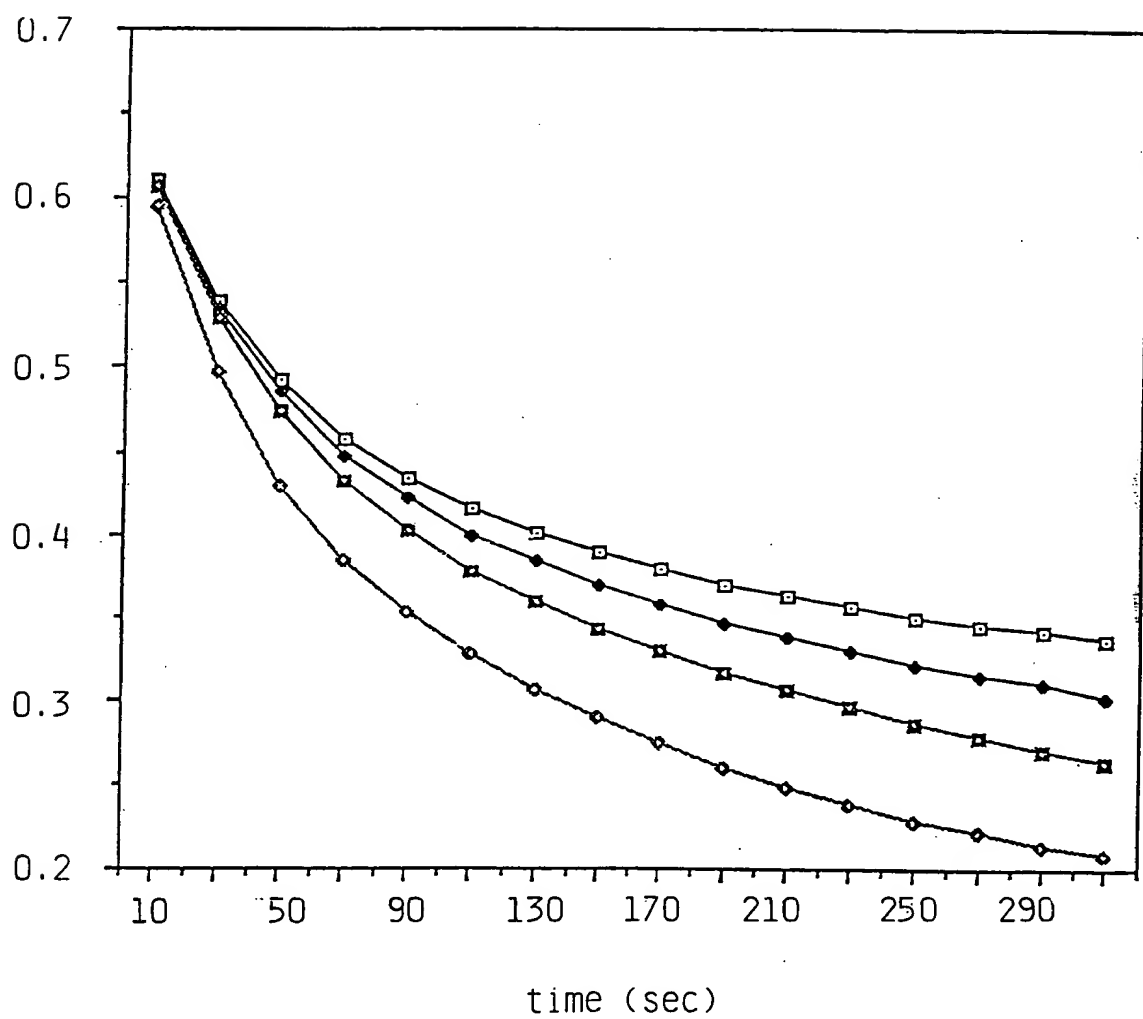


Fig. 2



## A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C12N 9/02, D21C 9/10, C11D 3/386

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: C12N, C11D, D21C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA, WPI, IFIPAT, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO, A1, 9105839 (NOVO NORDISK A/S ET AL), 2 May 1991 (02.05.91), see claims --	1-50
A	WO, A1, 9220857 (HANS-PETER HEINSBERGER), 26 November 1992 (26.11.92) -- -----	1-50

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

## \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

29 Sept 1994

Date of mailing of the international search report

11 -10- 1994

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**INTERNATIONAL SEARCH REPORT**  
 Information on patent family members

27/08/94

International application No.

PCT/DK 94/00210

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP-A,B- 0495836	29/07/92
		SE-T3- 0495836	
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		NO-D- 934148	00/00/00